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The Effects of Vitamin D Supplementation During Infancy on Growth During the First Two Years of Life

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Abstract

Context. The relationship between maternal and infant vitamin D and early childhood growth remains inadequately understood.

Objective. To investigate how maternal and child 25-hydroxyvitamin D [25(OH)D] and vitamin D supplementation impact growth during the first 2 years of life.

Design. A randomized, double-blinded intervention study.

Setting. A single-center study from pregnancy until offspring age 2 years.

Participants. Altogether 812 term-born children with complete data, recruited at Maternity Hospital.

Intervention. Children received daily vitamin D_3 supplementation 10 μg (Group-10) or 30 μg (Group-30) from age 2 weeks to 2 years.

Main outcome measures. Anthropometry and growth rate at age 1 and 2 years.

Results. Toddlers born to mothers with Pregnancy 25(OH)D >125 nmol/L were at 2 years lighter and thinner than the reference group with 25(OH)D 50-74.9 nmol/L (P<0.010). Mean 2-year 25(OH)D concentrations were 87 nmol/L in Group-10 and 118 nmol/L in Group-30 (P<0.001). When Group-30 was compared with Group-10, difference in body size was not statistically significant (P>0.053), but Group-30 had slower growth in length and head circumference between 6 months and 1 year (P<0.047), and more rapid growth in weight and length-adjusted weight between 1 and 2 years (P<0.043). Toddlers in the highest quartile of 25(OH)D (>121 nmol/L) were shorter (mean difference 0.2 SD score (SDS), P=0.021), lighter (mean difference 0.4 SDS, P=0.001) and thinner (in length-adjusted weight) (mean difference 0.4 SDS, P=0.003) compared with the lowest quartile (<81.2 nmol/L).

Conclusion. Vitamin D and early childhood growth may have an inverse U-shaped relationship.

Introduction

Vitamin D has a vital role in childhood growth and development and chronic and severe vitamin D deficiency leads to rickets, stunted growth and delayed neuromuscular development (1). Maternal vitamin D deficiency may increase the likelihood of pregnancy complications and prenatal growth restriction (2–4). Vitamin D status is defined by blood 25-hydroxyvitamin D concentration [25(OH)D], which is generally considered sufficient at values at or above 50 nmol//L (1,5,6). Vitamin D insufficiency (25(OH)D below 50 nmol/L) is common worldwide (7,8), the prevalence ranging from 7% in Northern Europe to 90% in the Middle East (5). Especially populations with inadequate sunlight exposure are at an increased risk. In addition to endogenously produced vitamin D in the skin, diet and supplements are important sources of vitamin D.

A global consensus recommendation for prevention of vitamin D deficiency rickets was published in 2016 (1). The recommended vitamin D supplementation was a daily dose of 15 µg for pregnant women and 10 µg for infants (1). The latest guidelines given by the Finnish national health authorities in 2018 recommend a daily total intake of 10 µg for pregnant women and infants (9). WHO does not recommend vitamin D supplementation for pregnant women (10). Some researchers consider the target 25(OH)D level to be much higher than 50 nmol/L, preferably >75-100 nmol/L, and therefore recommend higher supplemental vitamin D intake, up to 100 µg/d, also for pregnant women (11–13).

In general, it is presumed that vitamin D enhances childhood growth, although evidence is limited and conflicting, and in particular the linearity of the relationship is not known (3,14–17). The association between 25(OH)D concentrations and growth might be non-linear and hence dissimilar at different ranges of 25(OH)D, which could explain discrepancies between studies.

The effect of vitamin D on childhood growth pattern may best be seen during the prenatal period and infancy due to rapid growth rate. Early growth is particularly relevant for later health outcomes as specific growth patterns have been associated with increased risk of chronic diseases, for example through body composition and metabolic changes. While the associations between prenatal growth

and adult disease are particularly well established (18), growth during the first years after birth is also important (19,20).

Vitamin D intervention in Infants (VIDI) –study is a double-blinded and randomized clinical trial (RCT) comparing the effect of daily vitamin D supplementation of 10 µg or 30 µg from birth until 2 years of age, the primary outcomes being bone strength and infection episodes (21,22). We previously reported an unexpected association between higher maternal and infant 25(OH)D with slower infant growth in the VIDI cohort; mothers with 25(OH)D above 125 nmol/L had the smallest infants at 6 months and 1 year of age (23). In the current study, we aimed to investigate if maternal and child 25(OH)D further predict growth parameters at 2 years of age, and whether the dose of vitamin D supplementation in infancy influences childhood growth pattern from 6 months to 2 years of age.

Materials and Methods

Subjects

In Helsinki, Finland (60th parallel North), we recruited at Kätilöopisto Maternity Hospital 987 families to the VIDI study between January 2013 and June 2014. A description of the recruitment and study protocol has been published previously (21,22). Briefly, according to the inclusion criteria, the mothers were of Northern European origin without regular medication and with singleton pregnancy. Exclusion criteria for the newborns were: nasal continuous positive airway pressure treatment or need for nasogastric tube > one day, intravenous glucose infusion, seizures, and duration of phototherapy > three days. The infants were born between 37 and 42 weeks of gestation with birth weights appropriate for gestational age (standard deviation score [SDS] between -2.0 and +2.0).

Infants were randomized to receive daily vitamin D_3 supplementation with either 10 µg [hereafter referred to as Group-10] or 30 µg [hereafter referred to as Group-30] from age 2 weeks to 2 years. The study included three study visits at the age of 6 months, 1 and 2 years, and retrospectively and prospectively collected questionnaires.

Written informed consent was obtained from the parents at recruitment. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Ethical approval was obtained from the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (107/13/03/03/2012). The project protocol is registered at ClinicalTrials.gov (NCT01723852).

Of the recruited 987 families, we excluded 12 who did not meet the inclusion criteria and 1 infant diagnosed with Rieger syndrome, leaving 974 study participants. Further, 126 children were excluded from the present analysis due to lacking data on length and additional 9 due to lacking data on 25(OH)D at 2 years' follow-up. This resulted in a final number of 812 study subjects. Number of subjects varies in some analyses and are presented in tables and figures.

Family data

Parental data were obtained from a self-administered baseline questionnaire, filled out after delivery, and from medical records. Parental heights (cm) and weights (kg) before pregnancy were standardized into sex-specific z-scores. Body mass index (BMI) was calculated (kg/m^2).

Parental education level was categorized into 'lower' and 'higher' education (lower =lower or upper secondary or post-secondary non-tertiary education/less than a bachelor degree, higher =first or second stage of tertiary education/at least a bachelor degree), according to the highest received degree of either parent. Parental smoking status was assessed before pregnancy and at infant age of 2 years and applied as a merged previous and current smoking status. Family income level was enquired with a questionnaire completed at infant age of 2 years.

Child anthropometrics

Birth size was measured by midwives according to standard procedures. The measurements were collected from birth records, and transformed to parity-, gestational age- and sex-specific standard deviation scores (SDS) based on national newborn body size curves (24). Infant weight (kg), length (cm) and head circumference (cm) were measured at 6 months and at 1 and 2 years' follow-up visits by a pediatrician or a research nurse. At 1 and 2 years, mid-upper-arm circumference (MUAC) (mm) was measured. Length was measured with a tabletop meter in a supine position, and weight with an

electronic scale (Seca®, Hamburg, Germany). Weight, length, length-adjusted weight and head circumference were expressed as SDS using age- and sex-specific national references (25) and considered normal when between -2.0 and +2.0 SDS. BMI at 2 years of age was calculated and together with MUAC, standardized into sex-specific z-score within the present study population.

Study compliance and duration of breastfeeding were determined based on prospectively collected study diaries. Average vitamin D intake from food at 1 year of age was calculated based on 3-day food records (26).

Biochemical analyses

We analyzed 25(OH)D concertation from maternal serum samples in early pregnancy, at birth from umbilical cord blood (UCB), and from infant serum samples at the age of 1 and 2 years using the IDS-iSYS fully automated immunoassay system with chemiluminescence detection (Immunodiagnostic Systems Ltd., Bolton, UK). Pregnancy samples were collected at prenatal clinics on average at gestational week 11 between June 2012 and February 2014 as part of the mothers' normal follow-up [hereafter referred to as Pregnancy 25(OH)D] (23). UCB for 25(OH)D measurement was obtained at birth (gestational weeks 37 to 42) between January 2013 and June 2014 [hereafter referred to as UCB 25(OH)D]. Maternal 25(OH)D refers to both Pregnancy and UCB 25(OH)D. Children's samples at 1 year follow-up were obtained between December 2013 and May 2015 [hereafter referred to as Infant 25(OH)D], and samples at 2 years follow-up between December 2014 and May 2016 [hereafter referred to as Toddler 25(OH)D].

Pregnancy serum and UCB plasma 25(OH)D were analyzed simultaneously and Infant and Toddler serum 25(OH)D in a separate series with intra-assay variation <7% for Pregnancy 25(OH)D and Infant/Toddler 25(OH)D, and <13% for UCB 25(OH)D. The quality and accuracy of the 25(OH)D analyses are validated on an ongoing basis by participation in the vitamin D External Quality Assessment Scheme (DEQAS, Charing Cross Hospital, London, UK). The method showed a ≤8% positive bias against NIST Reference Measurement Procedure. Detailed information on the 25(OH)D analysis has been previously reported (22).

Vitamin D sufficiency was defined as 25(OH)D ≥50 nmol/L (5,6) . Further, we used additional cut-off values for 25(OH)D, namely 75 nmol/L, which has been suggested to be a higher threshold value for bone health (11) , and 125 nmol/L, above which values have been related to health risks (5,6).

Statistical analyses

The normality of the variables was visually inspected, and statistical tests were chosen accordingly. Infant and family characteristics were reported as means, standard deviations, and percentages. Covariates were chosen based on literature and consistent association with several growth measures. Missing values of covariates were multiple imputed (5 imputations). The difference between intervention groups was examined with Independent-Samples T-test, Mann-Whitney U-test or Pearson Chi-Square test.

Growth rate, referred to here as conditional growth, was investigated by using the residuals from linear regression models in which body size SDS at each successive age was regressed on corresponding body size SDS at all earlier ages (27). These residuals indicate how much a measurement of body size at each time point differs from that predicted by the corresponding measurements at earlier time points.

We used univariate and multivariate linear and quadratic regression analysis to determine associations between 25(OH)D and growth measures. We show in the tables unadjusted model 1, and model 2 adjusted with corresponding birth size, maternal and paternal height z-scores and intervention group. All analyses were also stratified by intervention group and shown in relevant tables separately. Additional adjustments were conducted with covariates of maternal prepregnancy BMI and paternal BMI, parental smoking status, parental education level, family income level, and duration of breastfeeding. These results are reported in the text only if an effect was observed.

Further, we investigated child growth in categories of 25(OH)D with ANCOVA adjusted for corresponding birth size SDS, maternal and paternal height z-scores and intervention group. Maternal 25(OH)D concentrations were categorized into four groups; <50 nmol/L, 50-74.9 nmol/L (reference

group), 75-125 nmol/L and >125 nmol/L, and Toddler 25(OH)D into three groups; <75 nmol/L (reference group), 75-125 nmol/L and >125 nmol/L due to only five toddlers having a concentration below 50 nmol/L. In addition, Toddler 25(OH)D concentration was categorized into quartiles; <81.2 nmol/L (reference group), 81.2-99.2 nmol/L, 99.3-120.7 nmol/L and >121 nmol/L. Differences in child size between categories were compared with linear regression applying 50-74.9 nmol/L, <75 nmol/L or first quartile as a reference group. Additional adjustments were conducted with covariates of maternal prepregnancy BMI and paternal BMI, parental smoking status, parental education level, family income level, and duration of breastfeeding. These results are reported in the text only if an effect on the results was observed.

Statistical significance was determined at $P < 0.05$. All statistical analyses were conducted using the IBM SPSS program for Windows, version 25 (IBM, Chicago, IL, USA).

Results

Subject characteristics are shown in Tables 1 and 2 according to intervention groups. Infant 25(OH)D concentrations were higher at the age of 1 and 2 years in Group-30 compared with Group-10 but no difference was observed in mean values of body size parameters (22). However, when we compared mean conditional growth values indicating growth rate, we discovered that growth in length and head circumference were slower between 6 months and 1 year, but growth in weight and length-adjusted weight were accelerated between 1 and 2 years in Group-30 compared with Group-10 (Figures 1 and 2). Almost all subjects (>92%) had normal body size (measured values between -2.0 and +2.0 SDS) at all time points.

At 1 year, total ($r=0.56$, $p<0.001$) and supplemental vitamin D intake ($r=0.59$, $p<0.001$) correlated with Infant 25(OH)D. Similarly, supplemental vitamin D intake at 2 years correlated with Toddler 25(OH)D ($r=0.61$, $p<0.001$). To exclude the possibility that body size as such, by possible dilution or fat mass, affected how vitamin D intake was reflected in 25(OH)D concentration, we tested

interactions between supplemental vitamin D intake (compliance-based µg/day) and weight (kg) in all linear models, and no interaction was detected.

No linear relation existed between Maternal 25(OH)D and offspring body size at 2 years (Table 3). But the mothers whose Pregnancy 25(OH)D was above 125 nmol/L had lighter (measured in weight) and thinner (measured in length-adjusted weight, MUAC and BMI) children at 2 years of age compared with the reference group of children with Pregnancy 25(OH)D 50-74.9 nmol/L (Figure 3). A quadratic association was confirmed between Pregnancy 25(OH)D and the children's length-adjusted weight and BMI at 2 years ($p<0.003$) suggesting an inverse U-shaped association (Figure 3). Toddlers at 2 years of age with UCB 25(OH)D below 50 nmol/L at birth were taller than the reference group of 50-74.9 nmol/L (Figure 4) but this association was attenuated after adjustment for maternal prepregnancy BMI, paternal BMI, parental smoking status, parental education level, family income level, and duration of breastfeeding ($p=0.062$). Toddlers with UCB 25(OH)D above 125 nmol/L were thinner (in BMI) at 2 years compared with the reference group of 50-74.9 nmol/L (Figure 4). Higher Pregnancy 25(OH)D and UCB 25(OH)D associated with accelerated growth in head circumference at 2 years, while no association for other growth parameters was observed (Table 4).

We have previously reported that across the VIDI cohort, higher Infant 25(OH)D at 1 year associated with slower growth at 1 year in several growth parameters (23). In the present study, we stratified the results according to intervention group, and observed that linear associations between Infant 25(OH)D and growth measures disappeared in Group-10 but were enhanced in Group-30 (Table 5).

At 2 years in the whole cohort, higher Toddler 25(OH)D associated linearly with smaller body size in all other parameters except head circumference (Table 6). The association between Toddler 25(OH)D and length attenuated after full adjustment for maternal and paternal factors ($p=0.054$). After stratification these linear results at 2 years of age by intervention group, associations between Toddler 25(OH)D and growth measures attenuated for length and remained for weight, length-adjusted weight and BMI in both groups, while for MUAC the association disappeared in Group-10 and remained in Group-30 (Table 6).

In the whole cohort, a quadratic association was observed between Toddler 25(OH)D and head circumference ($p<0.035$) implying an inverse U-shaped association (Figure 5). Toddler 25(OH)D had no linear relation for conditional growth at 2 years (Table 4).

When comparing growth parameters in three groups of Toddler 25(OH)D, those with 25(OH)D above 125 nmol/L (highest group) were lighter (in weight) and thinner (in length-adjusted weight and BMI) compared with the reference group with 25(OH)D <75 nmol/L (Figure 5). Toddlers with 25(OH)D between 75 nmol/L and 125 nmol/L had larger head circumference than the reference group of <75 nmol/L (Figure 5). Figure 6 shows adjusted mean values for growth measures in quartiles of Toddler 25(OH)D. Children in the highest quartile of 25(OH)D (>121 nmol/L) were shorter (in length), lighter (in weight) and thinner (in length-adjusted weight and BMI) than the reference group in the lowest quartile (<81.2 nmol/L) (Figure 6).

Discussion

We examined the association of vitamin D in pregnancy and in early childhood with child growth in an RCT-based cohort in Northern Europe with low sunlight exposure. VIDI study is an intervention trial with >800 infants comparing the effect of vitamin D supplementation of 10 µg/d and 30 µg/d during the first 2 years of life.

The dose of vitamin D supplementation had little effect on early childhood growth, as the mean body size measures were similar in both intervention groups (22). However, growth in length and head circumference was slower between 6 months and 1 year but growth in weight and length-adjusted weight was more rapid between 1 and 2 years in Group-30 compared with Group-10. Almost all children were vitamin D sufficient (≥50 nmol/L) and 21% of the children had 25(OH)D above 125 nmol/L at 2 years. The possible effect of vitamin D on growth may be mediated through 25(OH)D concentration, as we observed that higher 25(OH)D in early pregnancy, at birth, and at 1 and 2 years of age associated with smaller body size in the offspring during 2 years' follow-up.

Previous studies on vitamin D and growth in early childhood have been inconclusive (15,16,28–30). We have previously reported an inverse association between both Maternal 25(OH)D and Infant 25(OH)D and growth measures at age 6 months and 1 year (23). These findings were obtained before the intervention code was opened and were based solely on measured 25(OH)D concentration. In line with a Danish study (28) and contrary to an Equadorian study (30), we now observed that higher Toddler 25(OH)D at age 2 years associated with smaller anthropometric growth parameters. However, at 1 year, the associations were not observed within Group-10 but were enhanced in Group-30. This is consistent with a non-linear relationship, implying that the effect of vitamin D dosage on growth would depend on the attained 25(OH)D. At 2 years, these inverse associations between 25(OH)D and most growth measures remained in both intervention groups. This might be explained by differing growth rates between intervention groups and time points. The intervention effect may be smaller at 2 years than at 1 year because other factors such as food intake, physical activity and

endocrine factors, especially growth hormone secretion, have a larger role in child growth after infancy.

We also applied both clinical cut-off values and quartiles for Toddler 25(OH)D at 2 years. These results demonstrated that toddlers with 25(OH)D above 125 nmol/L or 121 nmol/L were the shortest (in length), lightest (in weight) and thinnest (in length-adjusted weight and BMI) at 2 years of age. In addition to our previous findings (31,32), others have found unfavorable and non-linear relations between vitamin D and child health outcomes (33,34).

In longitudinal analysis, maternal 25(OH)D concentration in early pregnancy and at birth had no linear relation to offspring growth anthropometry at 2 years. However, mothers with 25(OH)D above 125 nmol/L in early pregnancy, had the lightest and thinnest children at age 2 years, suggesting that maternal 25(OH)D may affect infant growth until age 1 year but the effect diminishes thereafter, possibly due to catch-up growth (23,35,36). Furthermore, other factors at an older age possibly have a larger role than maternal 25(OH)D if it is in the "moderate range". In line with our findings, Christensen et al. found an inverse relation between UCB 25(OH)D and offspring leg length from age 1.5 to 3 years of age (37). Further, U- or J-shaped association have been suggested to exist between maternal 25(OH)D and prenatal growth (38,39). However, several studies have found no relation between maternal 25(OH)D and offspring postnatal growth (40–45).

Conflicting results between studies may be related to geographical and genetic differences, leading to e.g. variable response to vitamin D supplementation (46,47), and varying cut-offs applied for 25(OH)D. Furthermore, it may be that only severe vitamin D deficiency (<30 nmol/L) (36,48), and, as suggested by our results, high 25(OH)D (>125 nmol/L) impair childhood growth. Vitamin D supplementation without vitamin D deficiency and "moderate" 25(OH)D concentrations would therefore not show associations with growth parameters. In our study, both maternal and child's 25(OH)D concentrations were at exceptionally high level compared with many other study cohorts. This was due to widely used vitamin D supplementation during pregnancy and national vitamin D food fortification (49,50). In the VIDI cohort we have shown that genotype modifies individual's 25(OH)D and the response to vitamin D supplementation (47,51). This individual dose-response was

shown in Group-30 but not in Group-10 (47), which might explain why we in the current study did not observe a similar relation between both vitamin D supplementation and vitamin D concentration and growth.

Severe vitamin D deficiency leads to growth impairment. If vitamin D indeed has an inverse U-shaped association with early growth, the mechanism how high 25(OH)D could disturb normal growth is unclear. Its role as a plasma calcium regulator could be one possible pathway. If high 25(OH)D leads to high circulating 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], this enhances calcium and phosphate resorption from bone to increase plasma calcium levels, thus possibly impairing growth (52). Based on one study, maternal 25(OH)D would not increase $1,25(OH)_2D$ after 25(OH)D reaches the level of 100 nmol/L (53). However, many organs and tissues, like the growth plate, have the ability to produce $1,25(OH)_2D$ locally (54,55) and thus high 25(OH)D could lead to high local production of 1,25(OH)2D. In our cohort, at age 1 year, Infant 25(OH)D correlated with plasma calcium (56) and PTH concentrations (23). Furthermore, PTH levels were lower in Group-30 than Group-10 at age 1 year and 2 years. These observations suggest that vitamin D influenced the endocrine system. However, the intervention group did not affect measured bone parameters (22). Vitamin D may also affect growth-regulating hormones, e.g. insulin-like growth factor 1 (IGF-1) which may activate $1,25(OH)_2D$ production (57, 58) .

We have a large and homogenous sample of North-European subjects with longitudinal data from early pregnancy until child age of 2 years covering all seasons. Data were collected and processed in a standardized fashion in a single maternity hospital. However, subjects had more commonly a higher education and normal weight than nationally representative population. The small number of subjects having maternal 25(OH)D values in both extreme ends, and only few vitamin D insufficient children may have constrained our analyses. We applied multiple methods to discover the possible relation between vitamin D and childhood growth and adjusted for potential confounders. As we did not observe a direct effect of vitamin D supplementation but rather consistent associations between 25(OH)D and growth parameters, we cannot determine true direction of causality. However, interactions of absolute body size and vitamin D intake were not observed.

The debate about the optimal 25(OH)D level for health outcomes is still ongoing (59,60). Studies with subjects of high 25(OH)D concentrations are scarce (61), especially in geographical locations with limited solar radiation, hence our data with exceptionally high 25(OH)D values are of importance in gaining more understanding about the relationship between vitamin D and health.

Conclusion

In this large study, high maternal and child 25(OH)D concentrations were associated with delayed growth in 1- and 2-years old children, but infant vitamin D supplementation in itself had only a minor impact on growth measures. Our results imply that vitamin D may have an inverse U-shaped relation with childhood growth. Therefore, aiming for higher than sufficient 25(OH)D levels with high vitamin D dosages may have undesired consequences on child growth. The clinical relevance of our results, however, remains to be evaluated in future studies.

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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Figure 1 Mean (95% CI) values of conditional growth at 1 year of age, i.e. growth rate, according to intervention groups. Conditional growth at 1 year refers to the difference of body size at 1 year with expected based on body size at birth and 6 months, expressed in standardized residuals, SD units. Statistical difference tested with Independent-Samples T test. Number of subjects for length and length-adjusted weight: 10 µg, n=401; 30 µg, n=410, for weight: 10 µg, n=402; 30 µg, n=410, and for head circumference: 10 µg, n=387; 30 µg, n=394.

Figure 2 Mean (95% CI) values of conditional growth at 2 years of age, i.e. growth rate, according to intervention groups. Conditional growth at 2 years refers to the difference of body size at 2 years with expected based on body size at birth, 6 months and 1 year, expressed in standardized residuals, SD units. Statistical difference tested with Independent-Samples T test. Number of subjects for length: 10 µg, n=401; 30 µg, n=410, for weight: 10 µg, n=401; 30 µg, n=409, for length-adjusted weight: 10 µg, n=400; 30 µg, n=409, and for head circumference: 10 µg, n=380; 30 µg, n=387.

Figure 3 Pregnancy 25(OH)D and offspring growth measures at 2 years of age. Symbols present adjusted mean (95% CI) values of growth measures in Pregnancy 25(OH)D categories of <50 nmol/L (n=24), 50-74.9 nmol/L (n=220) (reference group), 75-125 nmol/L (n=420) and >125 nmol/L (n=16). Adjustments are for corresponding birth size SDS, maternal and paternal height z-scores and intervention group. Statistical difference tested with linear regression with 50-74.9 nmol/L applied as a reference group. 25(OH)D, 25-hydroxy vitamin D; SDS, SD-score, based on Finnish sex- and age-specific normative data for infant growth; Length/weight, length-adjusted weight, HC, head circumference; MUAC, mid-upper-arm circumference (in z-score); BMI, body mass index (in z-score). The reference group's symbol has been highlighted.

Figure 4 Umbilical cord blood (UCB) 25(OH)D and offspring growth measures at 2 years of age. Symbols present adjusted mean (95% CI) values of growth measures in UCB 25(OH)D categories of <50 nmol/L (n=27), 50-74.9 nmol/L (n=304) (reference group), 75-125 nmol/L (n=429) and >125 nmol/L (n=34). Adjustments are for corresponding birth size SDS, maternal and paternal height z-scores and intervention group. Statistical difference tested with linear regression with 50-74.9 nmol/L applied as a reference group. 25(OH)D, 25-hydroxy vitamin D; SDS, SD-score, based on Finnish sex- and age-specific normative data for infant growth; Length/weight, length-adjusted weight, HC, head circumference; MUAC, mid-upper-arm circumference (in z-score); BMI, body mass index (in z-score). The reference group's symbol has been highlighted.

Figure 5 Toddler 25(OH)D and offspring growth measures at 2 years of age. Symbols present adjusted mean (95% CI) values of growth measures in Toddler 25(OH)D categories of <75 nmol/L (n=138) (reference group), 75-125 nmol/L (n=502) and >125 nmol/L (n=172). Adjustments are for corresponding birth size SDS, maternal and paternal height z-scores and intervention group. Statistical difference tested with linear regression with <75 nmol/L applied as a reference group. 25(OH)D, 25-hydroxy vitamin D; SDS, SD-score, based on Finnish sex- and age-specific normative data for infant growth; Length/weight, length-adjusted weight, HC, head circumference; MUAC, mid-upper-arm circumference (in z-score); BMI, body mass index (in z-score). The reference group's symbol has been highlighted.

Figure 6 Toddler 25(OH)D in quartiles and offspring growth measures at 2 years of age. Symbols present adjusted mean (95% CI) values of growth measures in Toddler 25(OH)D quartiles of 1. quartile (<81.2 nmol/L, n=203) (reference group), 2. quartile (81.2-99.2 nmol/L, n=203), 3. quartile (99.3-120.7 nmol/L, n=204) and 4. quartile (>121 nmol/L, n=202). Adjustments are for corresponding birth size SDS, maternal and paternal height z-scores and intervention group. Statistical difference tested with linear regression with 1. quartile applied as a reference group. 25(OH)D, 25-hydroxy vitamin D; SDS, SD-score, based on Finnish sex- and age-specific normative data for infant growth; Length/weight, length-adjusted weight, HC, head circumference; MUAC, mid-upper-arm circumference; BMI, body mass index. The reference group's symbol has been highlighted.

Table 1 Family characteristics according to intervention groups

	Group-10 n=402	Group-30 n=410	P value
Maternal age, year	31.4 (4.0)	31.9 (4.5)	0.10
Paternal age, year[a]	32.9 (5.0)	33.7 (5.8)	**0.026**
Maternal height, cm	166.3 (6.1)	166.3 (5.9)	0.94
Paternal height, cm[b]	180.7 (6.7)	180.2 (6.6)	0.36
Maternal prepregnancy BMI[c]	23.2 (3.7)	23.3 (3.7)	0.64
Paternal BMI[d]	26.0 (3.5)	25.6 (3.2)	**0.048**
Pregnancy 25(OH)D, nmol/L[e]	82.9 (21.9)	81.8 (17.8)	0.49
Pregnancy sampling, gestational week	11.3 (2.2)	11.5 (3.3)	0.39
Maternal supplemental vitamin D intake, µg/d[d]	17.1 (19.8)	14.6 (12.8)	0.26
Maternal smoking, yes, % (n)	15 (61/399)	16 (66/409)	0.74
Paternal smoking, yes, % (n)	26 (102/397)	25 (100/404)	0.76
Parental education, higher, % (n)	81 (322/397)	84 (345/410)	0.26
Family income level[f]			0.24
<40 000 €/year, % (n)	16 (64)	18 (70)	
40 000-89 000 €/year, % (n)	59 (231)	54 (216)	
>90 000 €/year, % (n)	20 (79)	20 (81)	
Don't know	5 (18)	8 (31)	

Values are means (SD) and P values are based on Independent-Samples T test, Mann-Whitney U test or Chi-Square.

[a]9 missing values; [b]16 missing values; [c]4 missing values; [d]24 missing values; [e]132 missing values; [f]22 missing values.

603
604
605 **Table 2 Infant growth parameters from birth to 2 years of age according to**
606 **intervention groups**

	Group-10 n=402	Group-30 n=410	P value
At birth			
Gestational age, wk	40.1 (1.1)	40.2 (1.1)	0.08
Length, cm	50.3 (1.7)	50.4 (1.8)	0.35
Length, SDS	-0.12 (0.89)	-0.11 (0.92)	0.86
Weight, kg	3.50 (0.37)	3.56 (0.40)	**0.027**
Weight, SDS	-0.19 (0.79)	-0.12 (0.84)	0.17
Length-adjusted weight, SDS	0.02 (0.93)	0.14 (0.93)	0.08
Head circumference, cm[a]	35.2 (1.4)	35.2 (1.4)	0.45
Head circumference, SDS[a]	0.19 (1.04)	0.08 (1.02)	0.12
UCB 25(OH)D, nmol/L [range][b]	83.4 (28.2) [36.7-283.7]	81.8 (23.5) [37.8-229.0]	0.39
At 1 year of age			
Age at follow-up, y	1.00 (0.03)	1.00 (0.03)	0.93
Length, cm[a]	75.4 (2.6)	75.2 (2.5)	0.19
Length, SDS[a]	-0.49 (1.0)	-0.59 (0.98)	0.14
Weight, kg	9.8 (1.2)	9.8 (1.1)	0.44
Weight, SDS	-0.19 (1.0)	-0.24 (0.99)	0.48
Length-adjusted weight, SDS[a]	0.04 (1.0)	0.04 (1.0)	0.94
Head circumference, cm[e]	46.6 (1.2)	46.4 (1.2)	0.08
Head circumference, SDS[e]	-0.32 (0.97)	-0.45 (0.93)	0.053
MUAC, mm[f]	152.7 (12.9)	153.0 (11.8)	0.69
MUAC[2], z-score[f]	-0.02 (1.0)	0.02 (0.96)	0.66
Blood 25(OH)D, nmol/L [range][g]	82.8 (19.9) [37.0-140.0]	116.0 (27.6) [51.8-241.0]	**<0.001**
Vitamin D intake from food, µg/day[h]	6.3 (3.7)	6.1 (3.7)	0.38
Compliance, %[i]	90.1 (10.4)	89.4 (10.6)	0.48
Supplemental vitamin D intake, compliance based µg/day[i]	9.0 (1.0)	26.8 (3.2)	**<0.001**
Energy intake, MJ/day[h]	3.36 (0.9)	3.31 (0.9)	0.26
At 2 years of age			
Age at follow-up, y	1.99 (0.03)	1.99 (0.03)	0.27
Length, cm	87.8 (3.2)	87.7 (3.0)	0.77
Length, SDS	-0.24 (1.04)	-0.27 (1.0)	0.71
Weight, kg[d]	12.5 (1.4)	12.6 (1.4)	0.44
Weight, SDS[d]	-0.19 (0.98)	-0.14 (0.99)	0.47
Length-adjusted weight, SDS[d]	-0.12 (0.98)	-0.02 (0.98)	0.15
Head circumference, cm[j]	49.1 (1.3)	49.1 (1.3)	0.60
Head circumference, SDS[j]	-0.22 (1.0)	-0.26 (0.98)	0.56
MUAC, mm[k]	161.8 (11.2)	162.5 (12.2)	0.42
MUAC, z-score[k]	-0.03 (0.95)	0.03 (1.04)	0.40
BMI, kg/m^2 [d]	16.2 (1.2)	16.3 (1.2)	0.15
BMI, z-score[d]	-0.05 (0.99)	0.05 (1.01)	0.14

Blood 25(OH)D, nmol/L [range]	86.5 (19.7) [42.4-153.5]	117.7 (26.1) [56.5-207.4]	**<0.001**
Compliance, %[l]	86.6 (16.2)	85.5 (17.7)	0.73
Compliance based supplemental vitamin D intake, µg/day[l]	8.7 (1.6)	25.6 (5.3)	**<0.001**
Duration of breastfeeding, months[m]	10.7 (5.7)	11.0 (5.6)	0.44

Values are means (SD). P values are based on Independent-Samples T test or Mann-Whitney U test. SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; UCB, umbilical cord blood; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference; BMI, body mass index.

[a]1 missing value; [b]18 missing values; [c]22 missing values; [d]2 missing values; [e]7 missing values; [f]34 missing values; [g]58 missing values; [h]107 missing values, breast milk intake not included; [i]10 values missing; [j]14 missing values; [k]17 missing values; [l]20 values missing; [m]6 values missing, duration of breastfeeding was set to 2 years if still ongoing at 2 years' follow-up

Table 3 Associations between Maternal 25(OH)D concentrations and offspring's growth measures at 2-years' follow-up visit

	SDS				z-score	
Pregnancy 25(OH)D, 10 nmol/L, n=680	Length	Weight	Length-adjusted weight	Head circumference	MUAC	BMI
Model 1, unadjusted	-0.01 (-0.05, 0.02)	-0.02 (-0.06, 0.02)	-0.02 (-0.05, 0.02)	-0.00 (-0.04, 0.04)	-0.03 (-0.07, 0.01)	-0.02 (-0.05, 0.02)
P value	0.48	0.29	0.38	0.97	0.12	0.42
Model 2, adjusted[a]	-0.02 (-0.05, 0.02)	-0.02 (-0.06, 0.01)	-0.00 (-0.00, -0.00)	0.00 (-0.02, 0.02)	-0.03 (-0.07, 0.00)	-0.02 (-0.05, 0.02)
P value	0.29	0.16	0.33	0.97	0.11	0.37
UCB 25(OH)D, 10 nmol/L, n=794						
Model 1, unadjusted	0.01 (-0.02, 0.03)	-0.01 (-0.04, 0.02)	-0.02 (-0.05, 0.01)	-0.01 (-0.04, 0.02)	0.00 (-0.02, 0.03)	-0.02 (-0.05, 0.00)
P value	0.67	0.43	0.13	0.40	0.80	0.09
Model 2, adjusted[a]	0.00 (-0.02, 0.03)	-0.01 (-0.03, 0.02)	-0.02 (-0.04, 0.01)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.02)	-0.02 (-0.04, 0.01)
P value	0.72	0.60	0.24	0.69	0.65	0.19

Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression.
SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference; BMI, body mass index; UCB, umbilical cord blood.
[a]Model 2 is adjusted for the corresponding birth size SDS (except for MUAC and BMI; the covariate was length-adjusted birth weight), maternal and paternal height z-scores, and intervention group.
Missing values: in Pregnancy 25(OH)D analyses: 1 value missing in weight, length-adjusted weight and BMI; 12 values missing from head circumference and MUAC, in UCB 25(OH)D analyses: 2 values missing in weight, length-adjusted weight and BMI; 15 values missing from head circumference and MUAC.

Table 4 Associations between Maternal and Toddler 25(OH)D and conditional growth at 2 years' follow-up visit

622

	SD unit				
Pregnancy 25(OH)D, 10 nmol/L, n=679	Length	Weight	Length-adjusted weight	Head circumference	MUAC
Model 1, unadjusted	-0.01 (-0.05, 0.03)	0.00 (-0.04, 0.03)	0.01 (-0.03, 0.04)	0.04 (0.00, 0.08)	0.00 (-0.04, 0.03)
P value for linear association	0.64	0.83	0.78	**0.036**	0.83
Model 2, adjusted[a]	-0.01 (-0.05, 0.03)	0.00 (-0.04, 0.03)	0.01 (-0.03, 0.04)	0.04 (0.00, 0.08)	0.00 (-0.04, 0.03)
P value for linear association	0.65	0.81	0.74	**0.037**	0.80
UCB 25(OH)D, 10 nmol/L, n=793					
Model 1, unadjusted	0.02 (-0.01, 0.04)	0.00 (-0.03, 0.02)	-0.02 (-0.04, 0.01)	0.03 (0.01, 0.06)	0.01 (-0.01, 0.04)
P value for linear association	0.23	0.74	0.20	**0.015**	0.30
Model 2, adjusted[a]	0.01 (-0.01, 0.04)	-0.01 (-0.03, 0.02)	-0.02 (-0.04, 0.01)	0.03 (0.01, 0.06)	0.02 (-0.01, 0.04)
P value for linear association	0.27	0.61	0.19	**0.014**	0.25
Toddler 25(OH)D at 2 years, 10 nmol/L, n=811					
Model 1, unadjusted	0.01 (-0.01, 0.04)	0.02 (0.00, 0.05)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	-0.01 (-0.03, 0.02)
P value for linear association	0.40	0.072	0.34	0.30	0.59
Model 2, adjusted[a]	0.00 (-0.03, 0.03)	0.01 (-0.02, 0.04)	0.00 (-0.03, 0.03)	0.01 (-0.02, 0.04)	-0.02 (-0.05, 0.01)
P value for linear association	0.82	0.60	0.82	0.48	0.32

623

Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression. Conditional growth refers to the difference of body size at 2 years with expected based on body size at birth, 6 months and 1 year, expressed in standardized residuals, SD units.

25(OH)D, 25-hydroxyvitamin D concentration; UCB, umbilical cord blood.

[a]Model 2 is adjusted for maternal and paternal height z-scores, and intervention group.

Missing values: in Pregnancy 25(OH)D analyses: 1 value missing in length-adjusted weight; 40 values missing from head circumference; 32 values missing from MUAC, in UCB 25(OH)D analyses: 1 value missing in length; 2 values missing length-adjusted weight; 42 values missing from head circumference; 44 values missing from MUAC, in Toddler 25(OH)D analyses: 1 value missing in length-adjusted weight; 2 values missing from length-adjusted weight; 44 values missing from head circumference; 46 values missing from MUAC.

Table 5 Associations between Infant 25(OH)D concentrations and growth measures at 1-year's follow-up visit stratified by intervention group

624

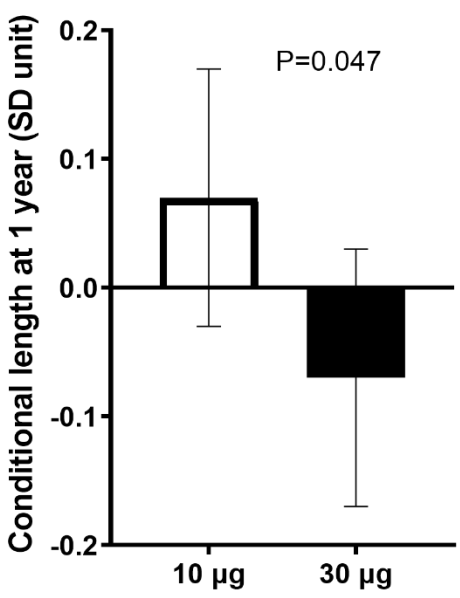
	SDS				z-score
Infant 25(OH)D, 10 nmol/L	Length	Weight	Length-adjusted weight	Head circumference	MUAC
Group-10, n=371					
Model 1, unadjusted	0.02 (-0.03, 0.07)	0.01 (-0.04, 0.06)	0.00 (-0.05, 0.05)	-0.01 (-0.06, 0.04)	-0.01 (-0.07, 0.04)
P value	0.47	0.69	0.95	0.79	0.68
Model 2, adjusted[a]	0.02 (-0.03, 0.07)	0.02 (-0.03, 0.07)	0.00 (-0.05, 0.06)	0.00 (-0.05, 0.04)	-0.01 (-0.06, 0.05)
P value	0.39	0.54	0.92	0.96	0.77
Group-30, n=383					
Model 1, unadjusted	-0.04 (-0.07, 0.00)	-0.07 (-0.10, -0.03)	-0.06 (-0.10, -0.02)	-0.04 (-0.07, 0.00)	-0.04 (-0.08, 0.00)
P value	**0.047**	**<0.001**	**0.001**	**0.039**	**0.026**
Model 2, adjusted[a]	-0.02 (-0.05, 0.01)	-0.05 (-0.09, -0.02)	-0.06 (-0.10, -0.02)	-0.02 (-0.05, 0.01)	-0.04 (-0.08, -0.01)
P value	0.17	**0.001**	**0.001**	0.19	**0.022**

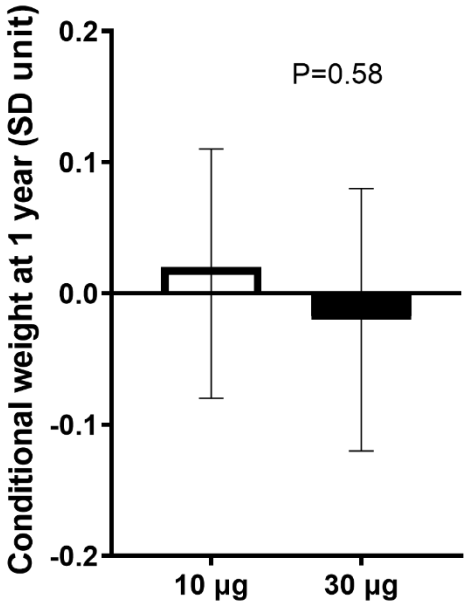
Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression.
SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference.
[a]Model 2 is adjusted for the corresponding birth size SDS (except for MUAC; the covariate was length-adjusted birth weight), maternal and paternal height z-scores and intervention group (except in analyses stratified by intervention groups).
Missing values: 1 value missing from length and length-adjusted weight; 5 values missing from head circumference; 32 values missing from MUAC.

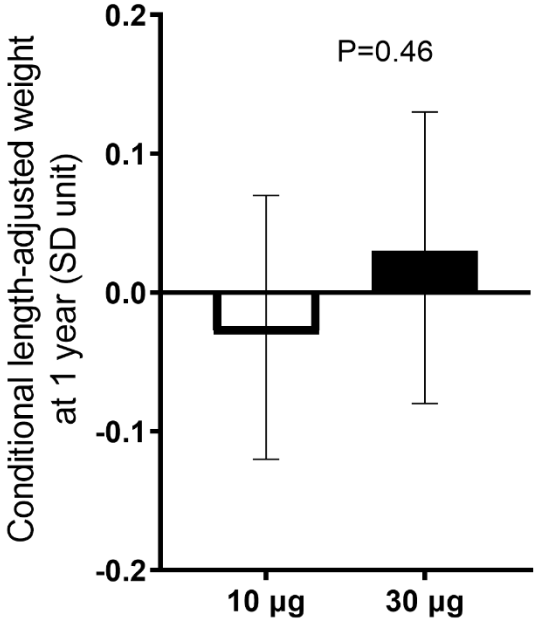
Table 6 Associations between Toddler 25(OH)D concentrations and growth measures at 2-years' follow-up visit stratified by intervention group

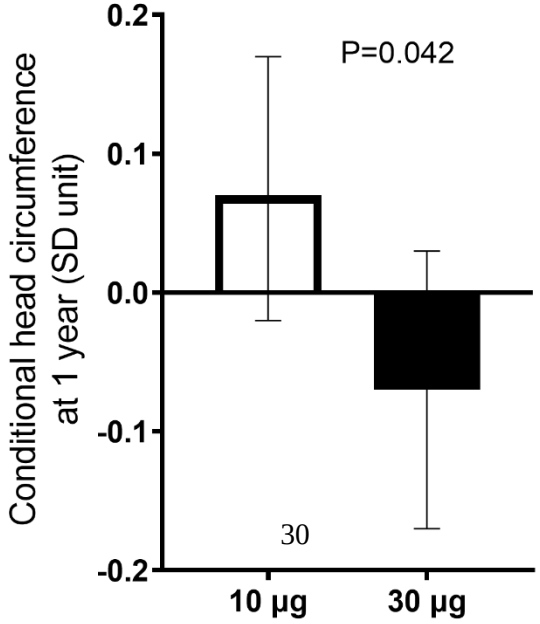
	SDS				z-score	
Toddler 25(OH)D, 10 nmol/L	Length	Weight	Length-adjusted weight	Head circumference	MUAC	BMI
All, n=812						
Model 1, unadjusted	-0.02 (-0.05, 0.00)	-0.03 (-0.06, -0.01)	-0.03 (-0.05, 0.00)	-0.01 (-0.04, 0.00)	-0.02 (-0.04, 0.01)	-0.03 (-0.06, 0.00)
P value	0.057	**0.009**	**0.040**	0.32	0.13	**0.035**
Model 2, adjusted[a]	-0.03 (-0.05, 0.00)	-0.04 (-0.07, -0.02)	-0.02 (-0.05, 0.00)	-0.00 (-0.02, 0.02)	-0.02 (-0.04, 0.01)	-0.06 (-0.09, -0.02)
P value	**0.038**[b]	**0.001**	**0.001**	0.92	**0.030**	**0.001**
Group-10, n=402						
Model 1, unadjusted	-0.04 (-0.09, 0.02)	-0.07 (-0.12, -0.02)	-0.06 (-0.11, -0.02)	0.02 (-0.04, 0.07)	-0.03 (-0.08, 0.02)	-0.06 (-0.11, -0.01)
P value	0.16	**0.008**	**0.009**	0.56	0.20	**0.011**
Model 2, adjusted[a]	-0.03 (-0.07, 0.01)	-0.05 (-0.10, -0.01)	-0.06 (-0.11, -0.01)	0.01 (-0.04, 0.06)	-0.02 (-0.07, 0.02)	-0.06 (-0.11, -0.01)
P value	0.19	**0.016**	**0.018**	0.68	0.33	**0.018**
Group-30, n=410						
Model 1, unadjusted	-0.03 (-0.07, 0.01)	-0.05 (-0.09, -0.01)	-0.04 (-0.08, -0.01)	-0.03 (-0.06, 0.01)	-0.04 (-0.08, -0.00)	-0.04 (-0.08, -0.01)
P value	0.12	**0.009**	**0.018**	0.15	**0.049**	**0.021**
Model 2, adjusted[a]	-0.02 (-0.06, 0.01)	-0.04 (-0.07, 0.00)	-0.04 (-0.06, -0.02)	-0.01 (-0.03, 0.01)	-0.04 (-0.06, -0.02)	-0.04 (-0.07, -0.01)
P value	0.13	**0.034**	**0.027**	0.56	0.062	**0.032**

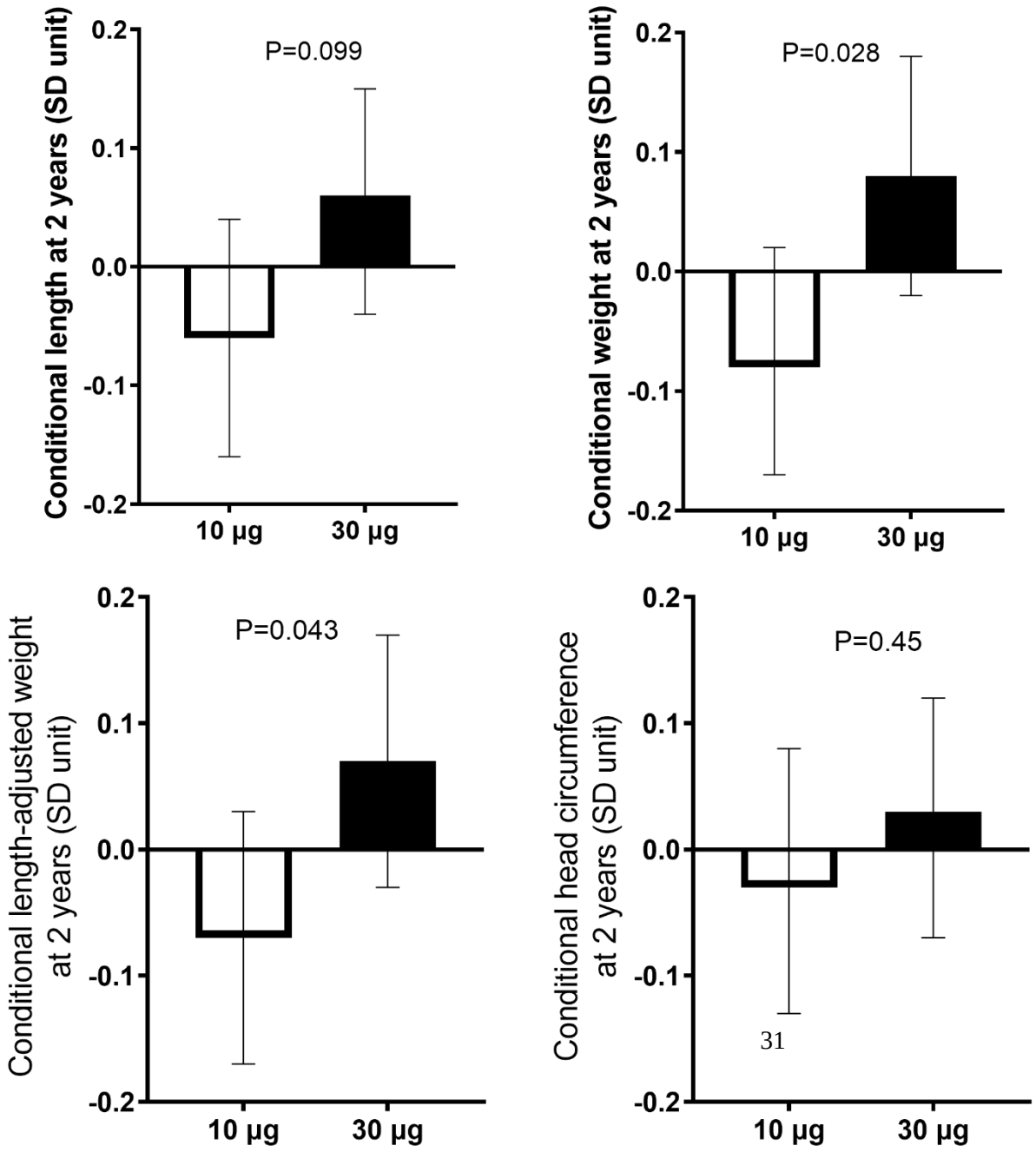
Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression.
SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference; BMI, body mass index.
[a]Model 2 is adjusted for the corresponding birth size SDS (except for MUAC and BMI; the covariate was length-adjusted birth weight), maternal and paternal height z-scores and intervention group.
[b]Additional adjustment for maternal prepregnancy BMI, paternal BMI, parental smoking status, parental education level, family income level, and duration of breastfeeding attenuated the association to P=0.054.
Missing values: 2 values missing from weight, length-adjusted weight and BMI; 14 values missing from head circumference; 17 values missing from MUAC.

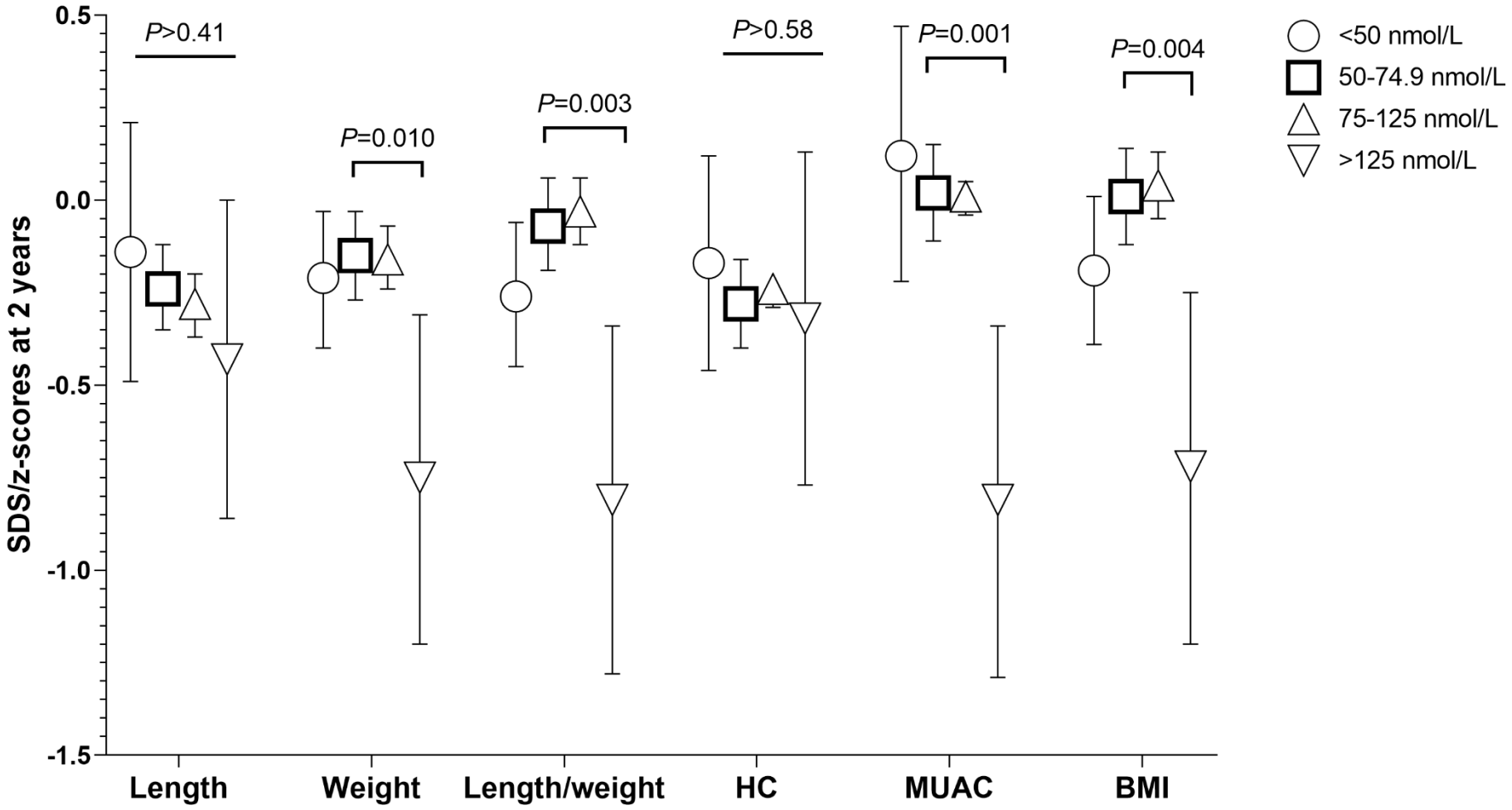

Conditional length at 1 year (SD unit)
P=0.047
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg

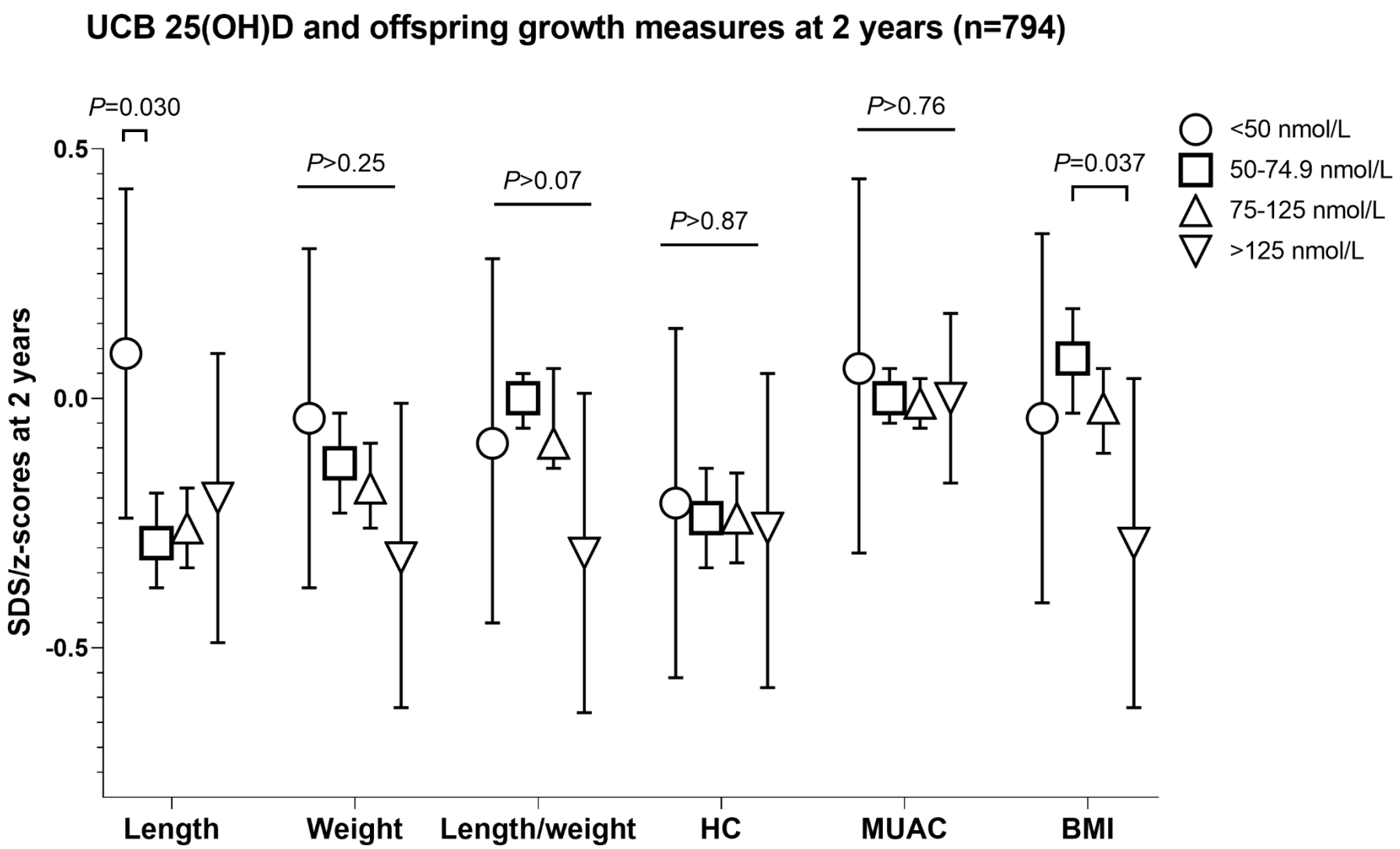

Conditional weight at 1 year (SD unit)
P=0.58
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg


Conditional length-adjusted weight at 1 year (SD unit)
P=0.46
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg

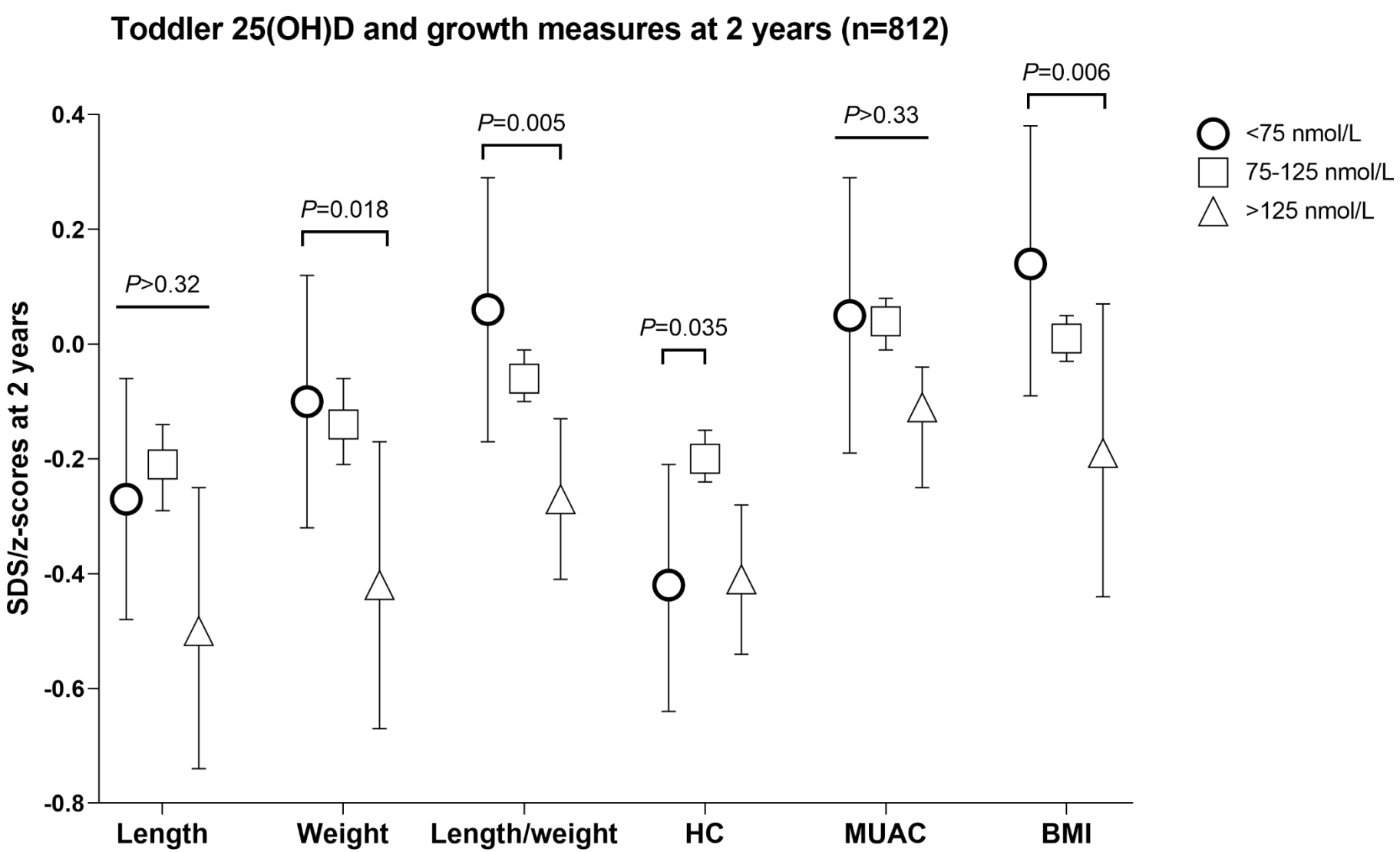

Conditional head circumference at 1 year (SD unit)
P=0.042
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg

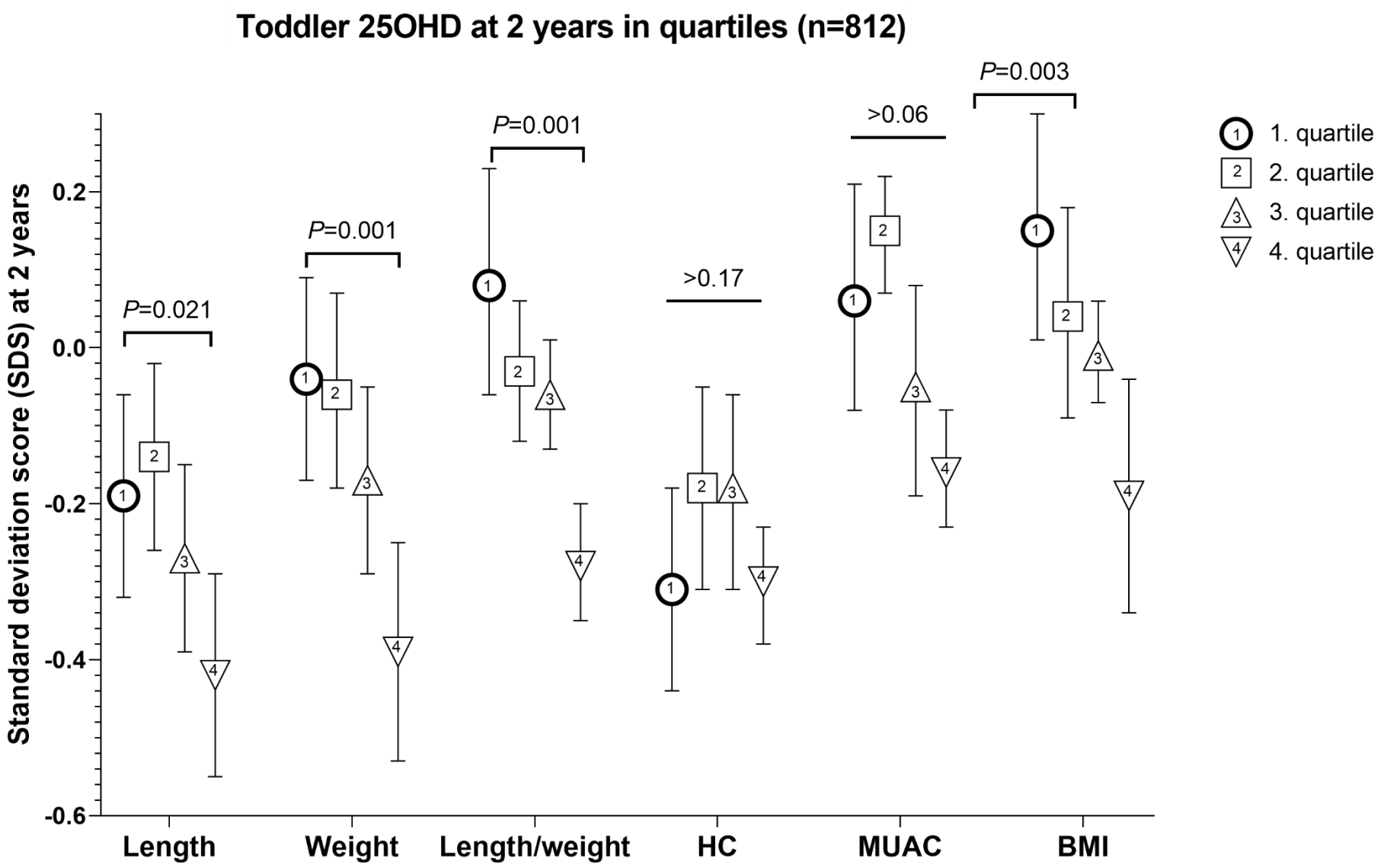

Conditional length at 2 years (SD unit)
P=0.099
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg
Conditional weight at 2 years (SD unit)
P=0.028
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg
Conditional length-adjusted weight at 2 years (SD unit)
P=0.043
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg
Conditional head circumference at 2 years (SD unit)
P=0.45
0.2
0.1
0.0
-0.1
-0.2
10 µg
30 µg


Pregnancy 25(OH)D and offspring growth measures at 2 years (n=680)
SDS/z-scores at 2 years
0.5
0.0
-0.5
-1.0
-1.5
P>0.41
P=0.010
P=0.003
P>0.58
P=0.001
P=0.004
Length
Weight
Length/weight
HC
MUAC
BMI
<50 nmol/L
50-74.9 nmol/L
75-125 nmol/L
>125 nmol/L


UCB 25(OH)D and offspring growth measures at 2 years (n=794)
P=0.030
P>0.25
P>0.07
P>0.87
P>0.76
P=0.037
<50 nmol/L
50-74.9 nmol/L
75-125 nmol/L
>125 nmol/L
SDS/z-scores at 2 years
0.5
0.0
-0.5
Length
Weight
Length/weight
HC
MUAC
BMI

625


Toddler 25(OH)D and growth measures at 2 years (n=812)
SDS/z-scores at 2 years
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
P>0.32
P=0.018
P=0.005
P=0.035
P>0.33
P=0.006
Length
Weight
Length/weight
HC
MUAC
BMI
<75 nmol/L
75-125 nmol/L
>125 nmol/L


Toddler 25OHD at 2 years in quartiles (n=812)
Standard deviation score (SDS) at 2 years
0.2
0.0
-0.2
-0.4
-0.6
P=0.021
P=0.001
P=0.001
>0.17
>0.06
P=0.003
Length
Weight
Length/weight
HC
MUAC
BMI
1. quartile
2. quartile
3. quartile
4. quartile